

Research Report

Expression of matrix metalloproteinase-8 gene in fixed orthodontic patients

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ABSTRACT

Background: Orthodontic treatment with fixed appliance produces structural and biochemical changes and breaking the balance between the synthesis and the breakdown of the collagen in the periodontium. Matrix metalloproteinase-8 (MMP-8) plays an important role in the remodeling of periodontal ligament during orthodontic movement. **Purpose:** The purpose of this study was to observe the expression of MMP-8 gene in the gingival crevicular fluid (GCF) of fixed orthodontic patients. It is expected that the result can be used as a reference to decide the proper time for elastomeric chain to be reactivated. **Methods:** Orthodontic fixed appliances were placed on 8 patients and elastomeric chains exerting 75 grams were attached to produce canine distalization. GCF samples were collected from the distal side of upper canines before force application, 1-, 2-, 3-, and 4 weeks after application consecutively. The samples were analyzed by using RT-PCR. Statistical analyses used were univariate analysis and Mann-Whitney U test. **Results:** The expression of MMP-8 in the GCF at t_0 was 31.3% but the force application elevated its expression to 65.6% at t_1 , and then decreased continuously at t_2 , t_3 , and t_4 . There was no statistically significant difference of MMP-8 gene expression between t_0 and t_3 . **Conclusion:** The highest level of MMP-8 gene expression due to orthodontic forces was occurred in the first week, but it declined continuously in the following weeks. The proper time to reactivate an elastomeric chain was 3 weeks after application.

Key words: MMP-8, fixed orthodontic appliance, reactivation time

ABSTRAK

Latar belakang: Perawatan ortodontik dengan peranti cekat menghasilkan perubahan-perubahan struktural dan biokimiawi pada jaringan periodontal dan mengganggu keseimbangan antara sintesis dan pemecahan kolagen pada periodonsium. Matrix metalloproteinase-8 (MMP-8) memainkan peran yang penting dalam remodeling ligamentum periodontal selama pergerakan gigi ortodontik. **Tujuan:** Tujuan dari penelitian ini ialah untuk mengamati ekspresi gen MMP-8 dalam cairan krevikuler gingiva (GCF) dari pasien ortodontik cekat. Diharapkan bahwa hasil penelitian ini dapat digunakan sebagai acuan untuk menentukan waktu yang paling tepat untuk mengaktifkan kembali rantai elastomer. **Metode:** Peranti ortodontik cekat dipasang pada 8 pasien dan rantai elastomer dengan kekuatan 75 gram dipasang untuk menarik gigi kaninus ke distal. Sampel GCF dikumpulkan dari bagian distal gigi kaninus atas berturut-turut sebelum aplikasi gaya, 1-, 2-, 3-, dan 4 minggu setelah aplikasi. Sampel dianalisis dengan menggunakan RT-PCR. Analisis statistik yang digunakan adalah analisis univariat dan uji Mann-Whitney U. **Hasil:** Ekspresi gen MMP-8 di dalam GCF pada t_0 adalah 31.3%, tetapi pemberian tekanan menaikkan ekspresinya menjadi 65,6% pada t_1 dan kemudian menurun secara kontinyu pada t_2 , t_3 , dan t_4 . Tidak ada perbedaan yang bermakna secara statistik antara ekspresi gen MMP-8 pada t_0 dan t_3 . **Kesimpulan:** Tingkat ekspresi tertinggi dari gen MMP-8 akibat tekanan ortodontik terjadi pada minggu pertama, tetapi kemudian menurun pada minggu-minggu berikutnya. Waktu yang paling tepat untuk mengaktifkan kembali rantai elastomer adalah 3 minggu setelah aplikasi.

Kata kunci: MMP-8, peranti ortodontik cekat, waktu reaktivasi

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INTRODUCTION

The main goal of orthodontic treatment is to obtain an optimal function of occlusion and facial aesthetics. Research and clinical observation showed that the treatment will be stable if there is a balance between the teeth and surrounding soft tissue. Along with the development of orthodontics, people who seek help to improve their irregular position of their teeth are increased. The need for orthodontic treatment increases not only in Indonesia but also in many other countries.¹⁻³

The teeth will move if subjected to pressure, followed by changes in the connective tissue. In the past, orthodontic resorption process is related to the pressure side and the apposition process on the strain side. Along with the development of science and technology, new discoveries have shown that bone tissue remodeling are seen from various sciences and clinical disciplines, which are all useful to human beings. Recent research showed that a perspective on orthodontic tooth movement based on molecular biology and immunology focused on the metabolic function of the extracellular matrix of periodontal tissues and bone, can be used to identify biological and diagnostic tools to monitor orthodontic tooth movement.⁴

The initial phase of orthodontic tooth movement usually involves many reactions resembling inflammation characterized by vascular changes and migration of leukocytes out of periodontal ligament capillaries. These changes lead to cellular activation and release of biologically active substances, such as enzymes and cytokines in the periodontal tissue.⁵

After the application of pressure, there will be structural and biochemical changes that disrupt the molecular balance between synthesis and degradation of collagen in the periodontal tissue. It showed that orthodontic tooth movement in humans, causing an increase in collagenase activity in gingival crevicular fluid (GCF). Matrix metalloproteinase (MMP) plays a very important role in periodontal tissue remodeling.⁶

MMP-8 hydrolyzes most effectively to collagen type I and III which are the major interstitial collagenases in human gingival inflammation.⁷ It has been demonstrated that the expression of MMP-8 and MMP-13 mRNA in periodontal ligament of rats was increased during active movement of teeth. Morphological and histo-chemical changes of periodontal ligament cells have been studied, but only few studies on MMP expression on the periodontal tissue due to mechanical pressure.⁸ Is it possible to observe the expressions of MMP-8 gene gingival crevicular fluid and used them as a reference to determine the proper time for elastomeric chain to be reactivated.

Therefore of this study, the aim was to investigate the expression of MMP-8 gene in gingival crevicular fluid during orthodontic tooth movement on fixed appliance wearers. It is expected that the result can be used as a

reference to decide the proper time to re-activate fixed appliance, in this case, elastomeric chain.

MATERIALS AND METHODS

Informed consent from the subjects was obtained after an explanation of the study protocol, which was reviewed by Ethical Committee of Medical Faculty at Hasanuddin University, Makassar. Eight adult orthodontic patients (two males, six females, aged 19-25 years \pm 2, 3 years) were enrolled in the study. All patients were treated at an academic orthodontic clinic, RSGMP-FKG Unhas, Makassar. They all had fixed appliances [Mini Roth bracket, 0.018 inch slot with elastomeric chain (Ormco, USA)] that gave light forces of approximately 75 grams. Inclusion criteria for sample selection are as follows: patients were suffering from maxillary protrusion and/or anterior crowding, based on Kesling's space analyses, they required premolar extraction, good oral hygiene, no periapical/periodontal diseases, no root anomaly in terms of shape and length, never undergone orthodontic treatment, and use no medication. Patients with the following conditions were excluded: suffering from systemic diseases (diabetes mellitus), extreme position of the canines, and gingival crevicular fluid mixed with blood.

The experimental design of this study is clinical follow-up study. In each subject, the upper first premolars were extracted before placing the brackets and wire. The canine undergoing distal movement was used as the experimental tooth. Orthodontic appliances were placed using an edgewise technique, in which 0.018 x 0.025-inch slot bands and brackets (Ormco, USA) were used. The canines were retracted with elastomeric chain (Ormco, USA) on a 0.018-inch round wire (Ormco, USA).

The canines were moved distally using an elastomeric chain that exerted an initial force of 75 g. At the distal aspect of the canines, GCF samples were collected before, and 1, 2, 3, and 4 weeks after initiation of tooth movement.

GCF samples were collected from distal sides (resorption sides) of gingival crevices of upper canines that orthodontically moved. The teeth at the sampling sites were isolated with cotton rolls and gently dried with air. Two paper points were carefully inserted into the gingival crevice and allowed to remain there for one minute but then discarded. Care was taken to avoid mechanical injury. The same method was repeated, but the paper points were placed into tubes with the buffered solution (L-6) insides. The samples were then frozen and kept at -20° until analysis.

The GCF samples were extracted to get a total RNA. The RT-PCR analysis was performed by putting the following reagents into a microfuge tube: 6 μ l reverse transcription buffer (Primecript, Takara, Japan), 1.5 μ l specific primer for MMP-8 i.e. sense primer: TGGACCCAATGGAATCCTTGC and antisense primer:

ATAGCCACTCAGAGCCCAGTA which generate 544-bp fragment, 1.5 µl enzyme mixt, 19.5 µl H₂O and 1.5 µl mRNA sample (it comes from 50 pg mRNA template). Then, the tube was incubated at 37° C for 15 minutes, it allows the reverse transcription to work. Raise the temperature to 94° C for 2 minutes, 60° C for 2 minutes, and 72° C for 3 minutes. DNA bands were observed after 37 cycles of PCR. glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was added to each sample served as an internal control/ house-keeping gene for the entire process. The PCR product was loaded onto 2% agarose gel for electrophoresis and visualized with UV light after gel incubation in ethidium bromide solution. The images were obtained by a digital camera.

The expression levels of MMP-8 relative to GAPDH can be scored through the photograph by three people which were previously calibrated. It was assumed that the higher concentration of RT-PCR product, the brighter the light of DNA band would be. They were expressed in a semi-quantitative score of 1–4 as follows: score 1 if the light of the DNA band was less bright than the control, score 2 if the light of the DNA band was the same bright with the control, score 3 if the light of the DNA band was slightly brighter than the control, and score 4 if the light of the DNA band was much brighter than the control.

The data obtained from the study were processed electronically using SPSS software version 15.0, then analyzed by using Mann-Whitney U statistical method and univariate analysis.

RESULTS

Results of this research consist of percentages of MMP-8 gene expression according to RT-PCR test of 40 samples from 8 subjects treated with fixed orthodontic appliances and determination of time in which the level of MMP-8 expression is same with the baseline's level using Mann-Whitney U test.

From the univariate statistical analysis, it showed that the gene expression of MMP-8 before the attachment of elastomeric chain was 31.3%. After the attachment, it was

up-regulated to 65.6 % in the first week, but then down-regulated to 56.3% in the second week, decreased again to 34.4% in the third week, and the lowest was 31.3% in the fourth week (Figure 1). The difference of MMP-8 gene expression between t_0 and t_2 was significant ($p = 0.003$). There was no significant difference between t_0 and t_3 ($p = 0.602$) (Table 1).

DISCUSSION

MMP is a member of group of enzyme that can break down protein, such as collagen, that are normally found in the spaces between cells of tissue i.e. extracellular matrix proteins. They are known to be involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as FAS ligand), and chemokine in/activation. MMPs are also thought to play a major role on cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis and host defense.^{10–12}

MMP-8 (collagenase-2), is a collagen cleaving enzyme which present in the connective tissue of most mammals. In human, the MMP-8 is encoded by MMP-8 gene. It is produced primarily by PMNs (polymorphonuclear cells) and released from the specific granules at sites of inflammation.¹³ Examination of the cells found in gingival crevicular fluid (GCF) has consistently shown that neutrophils constitute the largest number (about 92%) of cells.¹⁴ This was one of the reasons why GCF was used as a sample fluid to investigate the MMP-8 gene in the present study.

During orthodontic treatment with fixed appliances, a clinically healthy periodontium with no plaque or food debris accumulation is important. In the present study, all the patients had a clinically healthy periodontium. Some believe that the flow of GCF is induced by microbial accumulation at the dento-gingival junction. This flow increases greatly with inflammatory changes of gingivitis and periodontitis. The expression and activity of MMPs in adult tissues is normally quite low, but increases significantly in various pathological conditions that may lead into unwanted tissue destruction, such as periodontitis.¹⁵

Orthodontic treatment is mainly aimed at tooth movement by remodeling and adaptive changes in paradental tissue. To affect this outcome, only small amounts of force (20 to 150 g) per tooth might be required. It is assumed that an optimal force moves teeth efficiently into their desired position without causing discomfort or tissue damage to the patient.¹⁶ In this present study, an elastomeric chain exerting 75 g of force was used to move the upper canine distally.

Orthodontic tooth movement due to mechanical force of appliance can alter the PDL's vascularity and blood flow, resulting in local synthesis and release of various key molecules, such as neurotransmitters, cytokines, growth factors, colony-stimulating factors, and arachidonic

Table 1. Mann-Whitney U test result of the difference between $t_0 - t_2$ and $t_0 - t_3$ to the expression of MMP-8 gene in fixed orthodontic patients

Duration of force (week)	N	MMP-8 gene expression	Significance (p)
		Percentage (%)	
0	8	31.3	0.003
2	8	65.6	
0	8	31.3	0.602
3	8	34.4	

acid metabolites. These molecules can evoke many cellular responses by various cell types in and around teeth, providing a favorable microenvironment for tissue deposition or resorption.¹⁷ The levels of those mediators in GCF have been well demonstrated to be responsive to orthodontic force in humans.

It was also discovered that mechanical stresses alter the structural properties of tissues at the cellular, molecular, and genetic levels. However, only a few studies have been focused on the remodeling caused by MMP in GCF during orthodontic tooth movement.¹⁸

MMP-8 (collagenase 2) plays an important role in periodontal tissue remodeling. MMP-8 has ability to maintain the structure, integrity, and cellular activities and functions of the extracellular matrix of periodontal tissues. The main component of the extracellular matrix is tissue proteins, i.e. collagen, fibronectin, and glikosamino glikan. Orthodontic tooth movement causes a widespread degradation of collagen in the extracellular matrix of periodontal tissues and alveolar bone. It allows a release of cells from extracellular matrix environment, such as osteoblasts are moving into the apposition site and osteoclasts to the resorption site, causing a tooth movement.

The present *in vivo* study demonstrated (Figure 1) that the expression of MMP-8 gene at the baseline (t_0) was 31.3%, then the orthodontic force up-regulated sharply the MMP-8 gene expression to 65.6 % in the first week. It supported the result of previous study conducted by Apajalahti *et al.*,¹⁹ that MMP-8 level in the GCF significantly increased in the initial stage (at 4-8 hours from the application of fixed orthodontic appliance). Unfortunately, it was not reported when the MMP-8 level went down to the same level with that before force application. Ingman *et al.*,²⁰ in their study of MMP-1 and -8 in GCF during 1 month of follow-up after fixed appliance activation using IFMA method showed that the MMP-8 level was 12-fold higher than in control. In contrast with our present study, the level of MMP-8 on the fourth week was 2-fold higher than in the first week.

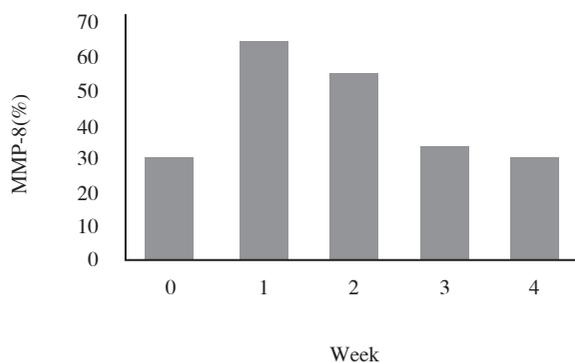


Figure 1. The percentages of MMP-8 gene expression according to RT-PCR test result, based on the duration of force.

In this present study, MMP-8 gene expression was also analyzed after long term (four weeks) tooth movement by using RT-PCR technique. The MMP-8 gene expression down regulated on the second week, more decreased on the third week and the least was on the fourth week. It can be assumed that the force induced by the elastomeric chain decreases with the time. As a consequence, the MMP-8 gene expression will decrease too.

In this study result, there was no difference significantly between the expression of MMP-8 gene before application (t_0) and that in the third week (t_3) (Table 1). It means that they had been more or less in the same level. At the time when the MMP-8 gene expression goes down to the same level with its expression before application, is assumed to be the proper time to reactivate the elastomeric chain.

It can be concluded that expression of MMP-8 gene in the GCF was up-regulated by the orthodontic pressure. The highest level of MMP-8 gene expression was happened in the first week, and then decreased gradually in the second, the third and the fourth week. The proper time to reactivate elastomeric chain was three weeks after application.

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